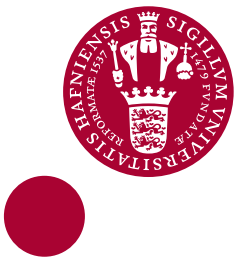

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Nitrogen – essential macronutrient and signal controlling flowering time

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Nitrogen, as limiting nutrient for plant growth and crop yield, is a main component of fertilizers and heavily used in modern agriculture. Early reports from over-application of fertilizers in crop production have shown to repress the transition from vegetative to reproductive phase. For the model plant *Arabidopsis thaliana*, there is evidence that low nitrogen conditions promote early flowering, while high nitrogen as well as nitrogen starvation conditions display the opposite effect. To gain a better understanding of how nitrogen affects the onset of flowering, we reviewed the existing literature for *A. thaliana* and carried out a meta-analysis on available transcriptomics data, seeking for potential genes and pathways involved in both nitrogen responses and flowering time control. With this strategy, we aimed at identifying potential gateways for integration of nitrogen signaling and potential regulators that might link the regulatory networks controlling nitrogen and flowering in *A. thaliana*. We found that photoperiodic pathway genes have high potential to be involved in nitrogen-dependent flowering.

Introduction

Nitrogen, the most abundant mineral in plants, is also one of the most limiting growth factors. Being part of all buildings blocks of life: nucleic acids, amino acids and proteins or metabolic products, nitrogen represents about 2% of plant dry tissue (Miller and Cramer 2005). Several specific strategies has evolved for nitrogen fixation and uptake in different plant families. However, the model plant *Arabidopsis thaliana* does not rely on extraordinary fixation strategies like nodulation or mycorrhiza, but is solely dependent on direct nitrogen uptake from the soil. Inorganic nitrogen is available either as ammonium (NH_4^+) or nitrate (NO_3^-), with the latter being especially abundant in agricultural soils (Crawford and Glass 1998). Nitrogen uptake is followed by assimilation and reduction of nitrate to nitrite and further to ammonium, catalyzed by nitrate (NR) and nitrite reductase (NIR), respectively. The assimilation of ammonium can take place in the root or in the shoot by photorespiration and ammonium is incorporated into organic molecules through the glutamine synthetase/glutamate synthetase (GS/GOGAT) pathway (Weber and Flügge 2002).

As nitrogen is crucial for growth, plants must be able to constantly measure nitrogen availability in their environment and respond accordingly. Beyond its role as nutrient, nitrate was recently described as a signaling molecule in plants (Bouguyon et al. 2012). Identified in first place as a dual affinity nitrate transporter (Tsay et al. 1993, Liu et al. 1999), later studies produced evidence for NRT1.1 being a nitrate sensor and signaling trigger, i.e. a transceptor, similar to nutrient sensing in yeast (Ho et al. 2009, Gojon et al. 2011). NRT1.1 can sense external nitrate concentrations and switch between

Abbreviations – Ca^{2+}, calcium; GA, gibberellin; GS/GOGAT, glutamine synthetase/glutamate synthetase; NIR, nitrite reductase; NR, nitrate reductase.

low- and high-affinity modes in an inverse fashion to external nitrate concentrations (Ho et al. 2009). Its affinity depends on the phosphorylation status of the threonine residue 101, whereas the phosphorylated form facilitates high- and the dephosphorylated form low-affinity transport (Liu and Tsay 2003). Moreover, NRT1.1 triggers a primary response after nitrate sensing, characterized by the induced expression of the high affinity nitrate transporter NRT2.1. Interestingly, the induction of the primary nitrate response is retained in *nrt1.1* mutants (e.g. *chl1–9*) lacking actual transport abilities, which suggests that nitrate transport and sensing is decoupled in NRT1.1 (Ho et al. 2009). In line with these results, it was shown that the phosphorylation status of NRT1.1 is important to regulate the expression of primary response genes after its initial induction (Bouguyon et al. 2015). Changes in gene expression triggered by nitrate and mediated by NRT1.1 are dependent on the second messenger Ca^{2+} (Riveras et al. 2015). Transcriptome studies revealed diverse nitrogen-regulated genes and pathways, supporting nitrogen as a signal (Undurraga et al. 2017). Consequently, nitrate shapes plant physiology and development in a broad manner from the induction of seed germination and regulation of root architecture to shoot development and the onset of flowering (Alboresi et al. 2005, Castro Marín et al. 2011, Vidal et al. 2014, Kiba and Krapp 2016, Yuan et al. 2016).

During their lifetime, plants undergo several fundamental developmental transitions; germination, a shift to an autotroph lifestyle, a juvenile to adult vegetative transition and finally, as in the case of *A. thaliana*, reaching the reproductive state. These transitions during development require precise timing, regulation and appropriate responses to environmental cues. This generates the need for tight management of plant metabolism including appropriate allocation of important macronutrients such as nitrogen. As a consequence, nitrogen has been associated with the regulation of all developmental phase changes, from the embryo to reproduction (Vidal et al. 2014). This review focuses on developmental gene networks influenced by nitrogen availability and sensing, in particular the transition from vegetative to reproductive growth, or how nitrogen shapes flowering time regulation in Arabidopsis.

Nitrogen, a regulatory element for timing of flowering

The timing of flowering is a pivotal event in annual plants; and it has consequences for fertility and population sustainability. The flowering time network has been classified to be controlled by five major genetical pathways, namely autonomous, endogenous aging, gibberellin (GA), photoperiodic and vernalization pathways (Srikanth and Schmid 2011) (Fig. 1). Environmental changes serve as information input to the network, and environmental conditions such as heat, drought, cold, salinity or high irradiation can alter flowering time. The responses to environmental changes typically vary between *A. thaliana* accessions with accelerated or decelerated flowering process, which can be interpreted as escape strategy into the next generation in the case of early flowering (Kazan and Lyons 2016, Takeno 2016). Changes in the overall nutritional status and nutrient availability can change onset of flowering in a species- and accession-dependent manner. Nitrogen limitation has been shown to modify the network by induction of early flowering (Srikanth and Schmid 2011), while *A. thaliana* plants grown under high nitrogen conditions flower later than plants grown under low conditions. Likewise, plants exposed to severe nitrogen starving conditions are delayed in development and also flower late (Lin and Tsay 2017). Especially in native soils, the levels of bioavailable nitrogen highly fluctuate in space and time. In agriculture soils, these fluctuation are also present, up to 10 m*M* nitrate is considered as average, whereas ammonium is less abundant with concentrations below 1 m*M* (Crawford and Glass 1998, Miller et al. 2007). Indeed, strictly defined laboratory standards for nitrogen sources and concentrations do not exist yet, which have resulted in many different choices made for starvation, sufficient, inhibitory and high nitrogen supply. Not only the amount, but also the kind of growth media influences the availability and effect of nitrogen concentration on plant growth, as recently reviewed regarding flowering time (Lin and Tsay 2017). Evidence for nitrate itself as the signal regulating flowering time was found when complementation of low nitrate with glutamine did not rescue the early flowering phenotype (Castro Marín et al. 2011).

Potential regulatory links between nitrogen signaling and the flowering network

No nitrogen pathway within the flowering network has been described yet, but an increasing number of studies seek potential genes involved in the integration of nitrogen availability/sensing/signaling into flowering time control. Testing prominent flowering time genes for their response to nitrogen represent a direct, targeted approach to decipher the regulatory interconnectivity between nitrogen signaling and the onset of flowering. Central elements of the flowering network such as CONSTANS (CO) and GIGANTEA (GI) modulate the flowering time through the photoperiodic and circadian pathway. In inductive long day conditions, both

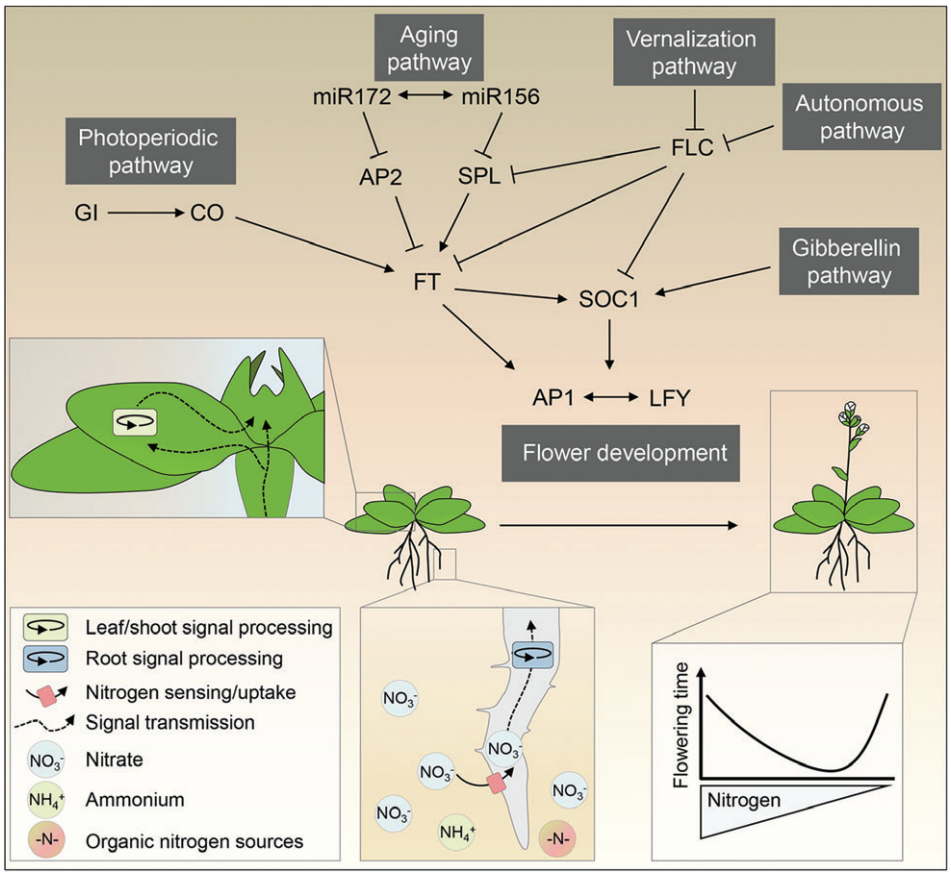

Fig. 1. Model of flowering time regulation in response to nitrogen. Crosstalk of the major flowering time pathways in *A. thaliana* and regulatory genes are further discussed in the text. Interconnected but distinct pathways (gray boxes) respond to environmental changes like day length or temperature and internal signals. The model represents a plant grown under nitrogen sufficient conditions.

proteins act as positive regulators of the expression of the florigen *FLOWERING LOCUS T (FT)*. Active FT protein travels to the SAM where it interacts with FLOWERING LOCUS D (FD) to induce meristematic key genes such *APETALA1 (AP1)*, *SUPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1)* and accordingly *LEAFY (LFY)*, thus inducing flower formation. Autonomous and vernalization pathways function through repression of *FLOWERING LOCUS C (FLC)* to induce flowering. Commonly, mutants of the autonomous pathway leading to elevated FLC levels, day length independent late flowering and the mutation is reversible by prolonged cold, known as vernalization. The MADS-box transcription regulator FLC inhibits the expression of floral integrators including FT, SOC1 and FD, among others. *FLC* in turn is downregulated after vernalization. In certain *A. thaliana* accessions, cold treatments trigger antisense transcription of the *FLC* locus, leading to *COOLAIR RNA* production and subsequent silencing of *FLC* transcription. FLC also act in the aging pathway through prolonged progression from juvenile to vegetative phase, caused by inhibition of SQUAMOSA PROMOTER-BINDING PROTEIN (SBP)-LIKE (SPL) transcription factors, SPL15 and SPL3 (Deng et al. 2011). In addition, the *flc-3* mutant enters the transition from juvenile to adult more rapidly, indicated by increased number of abaxial trichomes and early transition to the adult leaf shape (Mentzer et al. 2010, Willmann and Poethig 2011).

Developmental phase transitions are further coordinated by the action of the microRNA (miRNA) families miRNA156 and miRNA172. During aging, the abundance of miRNA156 declines constantly along with a gradual increase of its targets, the SPL transcription factor transcripts. SPLs increase during development accompanied by miRNA172, causing the posttranscriptional downregulation of the repressive APETALA2-like transcriptions factor family (AP2). Decrease in AP2 levels together with photoperiodic inductive long days lead to *FT* gene activation. The floral induction by the FD-FT complex is guided by SPL3/4/5 through binding an

upstream promotor region of the meristem genes *AP1*, *FRUITFULL (FUL/AGL8)* and *LFY* (Jung et al. 2016). In contrast, SPL9/15 mainly promote flowering through GA responses and in combination with induction of miR172 expression (Wu et al. 2009, Yu et al. 2012). The bioactive GA4 accumulates at the apical meristem shortly before the onset of flowering whereas the synthesis occurs in the leaves. Phytochromes mediate under photoperiodic control the upregulation of GA20ox resulting in increased GA concentrations in the leaves. GA transport into the shoot apical meristem is gated through the inactivation of GA by GA20x, constituting a fine tuning mechanism for GA induced activation of *SOC1* and *LFY* via the repression of the DELLA proteins GAI and RGA (Achard et al. 2004, Jasinski et al. 2005, Hisamatsu and King 2008). This illustrates that age-induced flowering is tightly connected with photoperiodic factors and GA signaling, orchestrated by posttranscriptional regulation.

Nitrogen nutrition modulates the basic flowering pathways

Castro Marín et al. (2011) showed that the flowering time response to different nitrate regimes (1, 10 or 35 m*M*, all supplemented with 4 m*M* glutamine) is stronger in mutants of photoperiodic (*co-2 and ft.-7*) and autonomous (*fwa-1, fve1 and fy-1*) pathways. Mutants of the GA pathway (*gai or ga1–3*) showed a trend, but no significant response. Furthermore, overexpression of CO and consequently constitutive activation of the photoperiodic pathway, as well as the inhibition of autonomous and vernalization pathways due to overexpression of FLC, abolishes the flowering response to nitrate, resulting in accelerated or elevated flowering time, respectively. Increased FLC expression in *fwa-1, fve1 and fy-1* is, however, not strong enough to prevent a *N*-regulated flowering response (Castro Marín et al. 2011).

The triple mutant *fca co-2 ga1-3*, blocked in photoperiodic, autonomous and GA pathway, reveals a severe phenotype and never flowers under long- or short-day conditions, yet, flowering can still be promoted by vernalization (Reeves and Coupland 2001, Castro Marín et al. 2011). Interestingly, low nitrate is also able to induce flowering in this triple mutant, which is not the case for other stresses like: high light, high temperature, photochilling or continuous light (Castro Marín et al. 2011).

Endogenous GA levels can increase or decrease upon low- and high-nitrate treatments (Liu et al. 2013) indicating a wide input spectrum for nitrogen into the flowering network. On the expression level, the flowering repressor *FLC* is repressed, while positive flowering integrators like *FT*, *LFY* and *AP1* are upregulated in low nitrate conditions (Kant et al. 2011). In addition, expression of *GA1* and *CO* is increased if plants are grown under limiting nitrate conditions. *SOC1*, an integrator of signals from multiple flowering pathways is upregulated as well (Liu et al. 2013). Taken together, nitrogen is influencing genes from autonomous, vernalization, GA and photoperiodic pathways.

Circadian clock, light perception, metabolic status and aging represent nitrogen targets

Recently, low nitrogen was reported to increase NADPH/NADP+ and ATP/AMP ratios following induction of *FERRODOXIN-NADP(+)-OXIDOREDUCTASE 1* (*FNR1*) expression (Yuan et al. 2016). The higher ATP/AMP ratios reduced nuclear *ADENOSINE MONOPHOSPHATE-ACTIVATED PROTEIN* Kinase (AMPK) activity and CRYTOCHROME 1 (CRY1) phosphorylation, stabilizing nuclear CRY1 abundance. In addition, mutant lines of *cry1* and *fnr1* are insensitive to changes in nitrogen availability and lack *N*-responsive flowering phenotypes. CRY1 is known to interact with SPA1 to suppress COP1-mediated degradation of CO in response to blue light (Liu et al. 2011). Concerning different nitrogen regimes, CRY1 seems to either enhance or decrease the expression amplitude of the core circadian genes *CIRCARDIAN CLOCK ASSOCIATED 1 (CCA1), LATE ELONGATED HYPOCOTYLE (LHY)* and *TIMING OF CAB EXPRESSION 1 (TOC1)*. The regulation of *CCA1* expression and phase shift trough changes in organic nitrogen (glutamate/glutamine) levels was previously identified and it was shown that CCA1 binds the promoters of the *N*-assimilatory genes *BASIC LEUCIN-ZIPPER 1 (bZIP1)*, *GLUTAMATE DEHYDROGENASE 1 (GDH1)* and *GLUTAMINE SYNTHETASE CYTOSOLIC ISOZYME 1–1 (GLN1)* towards a preferred glutamine metabolism (Gutiérrez et al. 2008). Together, these results link light perception, photoperiod and the circadian oscillator with *N*-related flowering control. Regarding the effect of day length, short days show the severest effect, but the *N*-responsive flowering phenotype is stable in day neutral and long days (Lin and Tsay 2017).

Under nitrogen starvation (0 m*M*) the aging pathway is rolled back, indicted by induction of miR156 and reduced miR172 expression levels (Pant et al. 2009, Liang et al. 2012, Fischer et al. 2013). Consequently, one of the miR156 targets, *SPL3* is also downregulated under nitrogen starvation, inhibiting the phase change to reproductive live style (Vidal et al. 2014). In addition, reduced miR172 levels will potentially lead to higher

Table 1. Key parameters of used datasets and related studies (Additional information for each gene identified and corresponding dataset included can be found in Table S1).

Data set/Factor	Sample tissue	Age	Media type	Studies
3 m*M* nitrate treatment after severe (0.3 m*M*) growth	Shoot	21 days	Rockwool	Bi et al. 2007
3 m*M* vs 0.3 m*M* nitrate	Shoot	21 days	Rockwool	
Nitrate-free vs nitrate-treatment (5, 10 or 15 m*M*) for 8 h	Root	42 days	Sand	Gutiérrez et al. 2007
10 days nitrate starvation	Shoot	35 days	Hydroponics	Krapp et al. 2011
10 days nitrate starvation	Root	35 days	Hydroponics	
2 days nitrate starvation	Shoot	35 days	Hydroponics	
2 days nitrate starvation	Root	35 days	Hydroponics	
2–10 days nitrate starvation	Shoot	35 days	Hydroponics	
2–10 days nitrate starvation	Root	35 days	Hydroponics	
Nitrate starvation	Seedling	10 days	MS	Liang et al. 2012
limiting (3 m*M*) KNO_3 vs sufficient (10 m*M*) KNO_3	Shoot	25 days	Soil	Peng et al. 2007
4 h 0.5 m*M* NH_4Cl/ KNO_3 treatment after 24 h N starvation	Root	12–14 days	Hydroponics	Ristova et al. 2016
4 h 1 m*M* KNO_3 treatment after 24 h N starvation	Root	12–14 days	Hydroponics	
4 h 1 m*M* NH_4Cl treatment after 24 h N starvation	Root	12–14 days	Hydroponics	
30 min + 3 m*M* KNO_3 vs starved	Seedling	9 days	Liquid culture	Scheible et al. 2004
3 h + 3 m*M* KNO_3 vs starved	Seedling	9 days	Liquid culture	
Full nutrition vs starved	Seedling	9 days	Liquid culture	
20 min 250 μ*M* KNO_3	Roots	10 days	Liquid culture	Wang et al. 2003
Normal to low nitrogen/before to after floral transition	Seedling	7 days	MS	Yuan et al. 2016
Normal to low nitrogen	Seedling	7 days	MS	

amounts of AP2-like transcription factors, delaying transition to reproductive phase through potential stronger repression of *FT*. In flowering time, prolonged nitrogen starvation results in delayed flowering, likely even to an arrest in development. Lin and Tsay recently described a U-shaped flowering response from nitrogen starvation to nitrogen superior growth conditions, showing the delaying effect of starving and excess amounts of nitrogen on flowering (Lin and Tsay 2017).

How to identify key factors in nitrogen-regulated flowering

Although nitrogen is well known to affect the onset of flowering time, the genes mediating crosstalk between the nitrogen and flowering time regulatory networks have remained elusive. We hypothesized that, building on the extensive knowledge of the flowering time networks, candidate genes linking nitrate sensing and flowering time control can be retrieved from already existing transcriptomics datasets. In order to conduct a meta-analysis to identify candidate genes involved in flowering time regulation and nitrogen responses and thereby complement targeted approaches focusing on one or few of the major hubs in the flowering network, we searched the literature for suitable datasets. The datasets that was included in the meta-analysis all used *A. thaliana* wild type plants (Columbia-0) as genetic background and covered a diverse range of nitrogen treatments (from starvation to high nitrogen stress) as well as different growth stages and developmental conditions (Table 1 and Table S1, Supporting information).

Transcriptomic data from nine studies including 20 conditional sets (Wang et al. 2003, Scheible et al. 2004, Bi et al. 2007, Gutiérrez et al. 2007, Peng et al. 2007, Krapp et al. 2011, Liang et al. 2012, Ristova et al. 2016, Yuan et al. 2016) were compared to 379 known flowering time genes obtained from the FLOweRing Interactive Database (Bouché et al. 2016). Out of these, 115 genes showed significant fold-changes in transcript abundance in response to the applied nitrogen treatment in at least one conditional set (Table 1). Analyzing those further, we found differently regulated genes belonging to eight different input pathways of the flowering network (Fig. 2A). The most strongly represented groups were the photoperiodic and autonomous pathways, together covering over 50%. Further analysis using the PANTHER classification of the molecular function (Mi et al. 2013) reveals an enrichment in genes associated with transcription factor activity, DNA and nucleic acid binding compared to the input search list (Fig. 2B). These findings are in line with the current knowledge on the flowering time regulation as outlined above. Nevertheless, the strong representation of the photoperiodic pathway and transcription factor genes is notable and narrows down the list of genes potentially linking nitrogen responses and flowering time.

In order to identify genes responding to nitrogen treatments across various conditions we restricted the candidate list to genes with a frequency ≥ 3, leading

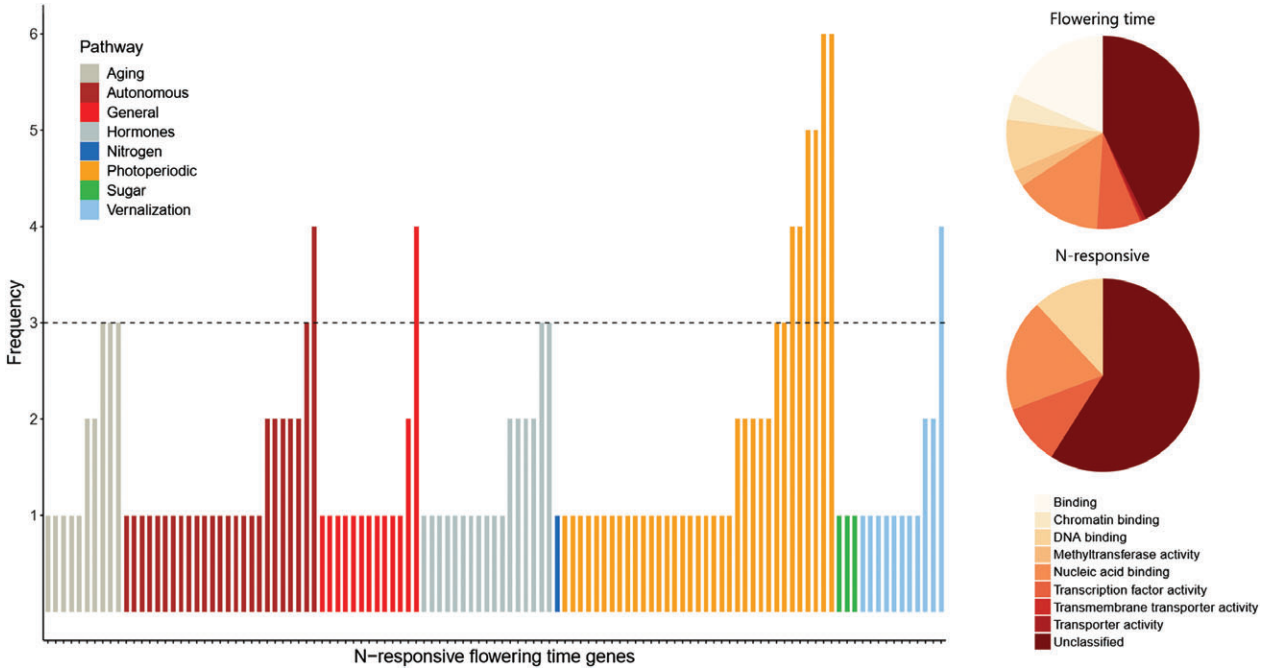

Fig. 2. Frequency plot of *N*-responsive flowering time genes. Expression data of designated flowering time genes was extracted from published array data (Table S1). Only significant ($P < 0.05$) and minimum two fold changed, if stated in the corresponding publication was included in the analysis. (A) Frequency of significantly changed flowering time genes. Genes are sorted according to pathways in the flowering time network and their frequency in array data. Dotted line indicates the threshold ≥ 3 for further analyses. (B) Analysis of functional categories was performed using PANTHER (version 11.1) with the *A. thaliana* PANTHER GO-Slim biological process annotation released 2016−10-24. Upper pie chart: Distribution of molecular gene functions in the flowering time input gene list (379 genes). Lower pie chart: Distribution of molecular gene function in the output gene list for N-responsive flowering time genes (by 115 genes).

to a final set of 17 genes (Figs-2A and 3). After this more stringent analysis, the genes from the photoperiodic pathway still represent the majority of the candidate genes. Strikingly, none of the major floral integrators like *CO*, *FT* and *SOC1* or the main repressor *FLC* is represented in the meta-analysis. Contrary, the peripheral pathway genes are responsive to nitrogen with the potential to influence the major hubs. The two genes that exhibited the highest frequency in our survey are *CONSTANS-LIKE 5 (COL5)* and *REVEILLE2 (REV2/CIR1)*. *REV2* is a MYB-related gene controlled by light and the central oscillator. Its expression is known to reduce the amplitude of *CCA1* and *LHY*, as well as the expression of *CO* and *FT*. Overexpression of *REV2* causes delay in flowering whereas the *cir1* knockout is early flowering (Zhang et al. 2007). In addition, *REV2* is a positive regulator of freezing tolerance (Guan et al. 2013). *REV2* shows a fast acceleration of expression upon nitrogen treatment and is downregulated under starvation (Table S1). Notably, *REV2* was only found in data sets using nitrate as the sole nitrogen source. However, our data landscape is dominated by nitrate treatments and is thereby biased towards genes responding to nitrate (Fig. 4B and Table S1). Assay parameters like plant tissue, age or day length do not seem to be critical for the response of *REV2* (Fig. 4). *COL5* is a *CONSTANT-LIKE* transcription factor, showing a *CO*-like circadian and diurnally regulated expression pattern. The overexpression of *COL5* can induce *SOC1* and *FT*, leading to early flowering without affecting *CO* (Hassidim et al. 2009). *COL5* is similarly to *REV2* downregulated under nitrogen starvation and increased after nitrogen application (Table S1). The nitrogen source, however, does not seem to be critical, as the *COL5* transcript respond to nitrate and ammonium treatments. In contrast, the exclusive detection of *COL5* in root sample is remarkable (Fig. 4), because gene expression maps indicate a low baseline expression in roots, except of increasing expression after cold treatments (Schmid et al. 2005). Further analysis revealed another interesting candidate gene, the *NUCLEAR FACTOR Y SUBUNIT A4* (*NF-YA4*), affiliated with the photoperiodic flowering pathway. *NF-YA4* exhibits the second highest frequency within the survey and shows a nitrate treatment-dependent response. Furthermore, *NF-YA4* is only present in shoot data sets from adult plants (Fig. 4). Interestingly, all three nitrogen-regulated genes, *REV2*,

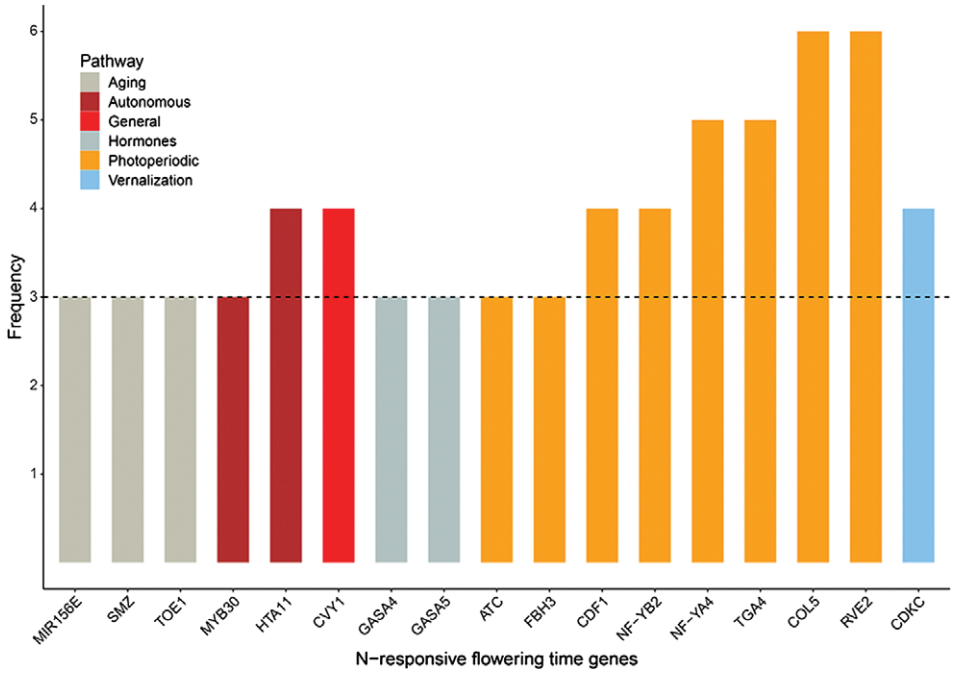

Fig. 3. Distribution of *N*-responsive genes enriched in the meta-analysis. *N*-responsive flowering time genes with a minimum frequency ≥ 3 (dotted line). Short names are given in the graph; for respective expression data, see Table1.

COL5 and *NF-YA4* have in common that they are cold inducible (Schmid et al. 2005). The fact that none of the major hubs from the flowering network was found is not surprising for several reasons. Their transcriptional responses to changes in nitrogen availability could be transient or masked by diurnal and developmental expression patterns. Moreover, many central regulators of flowering time are tightly regulated at both the transcriptional and post-translational level, but the relative contribution of the mechanisms in response to nitrogen treatments remains to be investigated. However, a fast response to changes in individual environmental factors does not meet the nature of a hub that initiates nonreversible developmental transitions, like the initiation of FT movement initiates floral transition in the meristem. Instead, regulatory hubs involved in developmental decisions need to integrate signals and feedback information from multiple pathways in order to provide the system with sufficient robustness.

Perspectives

The timing of flowering and responses to nitrogen nutrition are two major traits affecting plant reproductive success as well as productivity of crops. Both traits and their underlying pathways are under intensive investigation – mostly each by itself. As the induction or delay of flowering is a matter of nitrogen availability, as supported by experimental evidence (Castro Marín et al. 2011, Kant et al. 2011, Yuan et al. 2016, Lin and Tsay 2017), integrated studies of both traits must be strengthened to obtain a broader understanding. Thus, spatio-temporal aspects of nitrogen sensing and signaling will provide insight into flowering time control. The processing of the signal might take place in the root, where nitrogen is sensed by NRT1.1 or related NRT family members. Alternatively, direct transmission of either endogenous nitrogen or additional vasculature mobile signals might occur. Signal integration may take place directly in the shoot apical meristem as well as in the leaf, bypassing, e.g. the photoperiodic pathway. However, to elucidate the molecular mechanism mediating *N*-responsive flowering requires integrative omics studies under a broad range of defined conditions.

Author contributions

K. W. carried out the meta-analysis and drafted the manuscript. K. W. and M. B. commented on and edited the manuscript.

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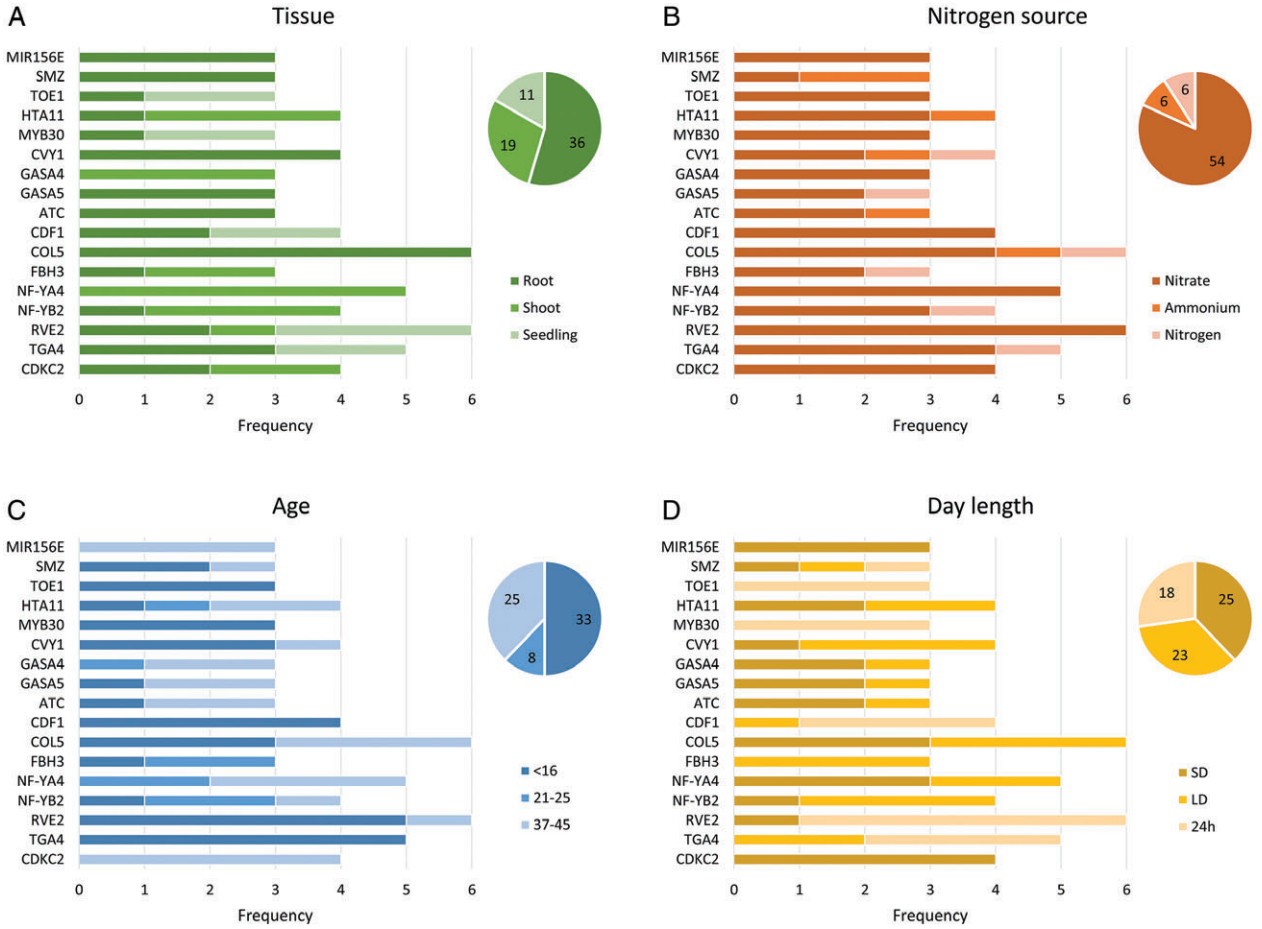

Fig. 4. Candidate genes in the context of the data landscape. Bar graphs show the distribution of genes with a frequency ≥ 3 across grouped experimental parameters. Pie charts depict the representation within each group of experimental parameters for all 66 observations of differential gene expression (17 candidate genes, 20 datasets). Additional details on the experimental setup for each dataset included can be found in Table S1. (A) tissue; (B) nitrogen source (Nitrogen: combined treatment of nitrate and ammonium); (C) plant age (days) at the point of harvesting for transcriptome analysis; (D) day length.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. N-responsive flowering time genes.

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